Typical Reaction Conditions for TEV Protease (NEB #P8112)

Overview

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Reactions may be scaled-up linearly to accommodate larger sample amounts and reaction volumes. Typical reaction conditions are as follows:

1. Combine 15 µg (0.2 nanomoles) of substrate and H_2O (if necessary) to make a 45 µl total reaction volume.
2. Add 5 µl of TEV Protease Reaction Buffer (10X) to make a 50 µl total reaction volume.
3. Add 1 µl of TEV Protease.
4. Incubate at 30°C for 1 hour or at 4°C overnight.

Notes:

1. If the fusion protein sample contains >2 M urea, >0.5 M Guanidine hydrochloride, >50 mM imidazole, pH values below 6 or above 9, or cysteine protease inhibitors then it will be necessary to dialyze the fusion protein into TEV protease reaction buffer before TEV Protease cleavage.
2. TEV protease is inhibited by reaction buffers containing >40% Glycerol.
3. Inhibition occurs in the presence of ≥ 5 mM Zn^{2+}, ≥ 1 mM Cu^{2+} and ≥ 10 mM Co^{2+}.
4. Compatible with 10mM $MgSO_4$, $MnCl_2$ and $CaCl_2$ and up to 100mM EDTA.
5. Compatible with the following protease inhibitors: aprotinin, benzamidine, leupeptin, pepstatin, PMSF.
6. Optimal activity achieved in ≤0.2M NaCl; however, the enzyme retains some activity in up to 2M NaCl.
7. Some substrates may require extended incubation periods (up to three days at either 4°C or 30°C) to achieve complete cleavage. The addition of more TEV Protease after 24 hours may also help achieve complete cleavage of some substrates.